

Attachment A

1. (original) A method for screening of a mutant lipase having an improved enzymatic activity comprising the following steps:
 - 1) Cloning a lipase gene into a surface display vector;
 - 2) Preparing a mutant lipase gene library by mutagenic PCR using the lipase gene in the surface display vector of the step 1 as a template;
 - 3) Transforming the mutant lipase gene library of the step 2 and the surface display vector fragment into host cells; and
 - 4) Measuring the activity of the mutant lipase displayed in the surface of the transformed host cell and selecting the lipase showing evolved activity.
2. (original) The method as set forth in claim 1, wherein the lipase gene of step 1 is *Candida antarctica* lipase B represented by SEQ. ID. No 14.
3. (original) The method as set forth in claim 1, wherein the surface display vector contains a promoter gene, a gene coding a secretion signal sequence, a lipase gene or a mutant lipase gene, a surface display mediating gene and a terminator gene.
4. (original) The method as set forth in claim 3, wherein the promoter gene is selected from a group consisting of GAPDH, PGK, ADH, PHO5, GAL1, GAL10, SED1, MOX, TEF and TPI.
5. (original) The method as set forth in claim 3, wherein the gene coding the secretion signal sequence is selected from a group consisting of MF α , PHO5, SUC2, AMY, SED and killer toxin.
6. (original) The method as set forth in claim 3, wherein the surface display mediating gene is selected from a group consisting of SED1, PIR2, TIP1, CWP1, GAS1 and WSC1.
7. (original) The method as set forth in claim 1, wherein the mutant lipase gene of step 2 is a gene coding a mutant protein in which #219 leucine and/or #278 leucine of *Candida antarctica* lipase B is replaced by other amino acids.

Attachment A

8. (original) The method as set forth in claim 1, wherein the host cell of step 3 is selected from a group consisting of yeasts such as *Candida*, *Debaryomyces*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Schizosaccharomyces*, *Yarrowia* and *Saccharomyces* genus, filamentous fungi such as *Aspergillus*, *Penicillium* and *Rhizopus* genus and bacteria such as *Escherichia* and *Bacillus* genus.
9. (original) The method as set forth in claim 8, wherein the host cell is a yeast cell selected from a group consisting of *Candida*, *Debaryomyces*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Schizosaccharomyces*, *Yarrowia* and *Saccharomyces* genus.
10. (original) A mutant lipase protein selected by the method of claim 1, in which #219 leucine and/or #278 leucine of *Candida antarctica* lipase B represented by SEQ. ID. No 14 is replaced by other amino acids.
11. (original) The mutant lipase protein as set forth in claim 10, wherein the #219 leucine is replaced by a hydrophilic amino acid selected from a group consisting of glutamine, histidine, arginine, lysine, serine, threonine, aspartic acid and glutamic acid.
12. (original) The mutant lipase protein as set forth in claim 11, wherein the #219 leucine is replaced by glutamine, and its amino acid sequence is represented by SEQ. ID. No 11.
13. (original) The mutant lipase protein as set forth in claim 10, wherein the #278 leucine is replaced by an amino acid selected from a group consisting of proline, tyrosine, phenylalanine, tryptophane and valine.
14. (original) The mutant lipase protein as set forth in claim 13, wherein the #278 leucine is replaced by proline, and its amino acid sequence is represented by SEQ. ID. No 9.
15. (original) The mutant lipase protein as set forth in claim 10, wherein the #219 leucine is replaced by glutamine, the #278 leucine is replaced by proline, and its amino acid sequence is represented by SEQ. ID. No 10.

Attachment A

16. (original) A gene coding the mutant lipase protein of claim 10.
17. (currently amended) The gene as set forth in claim 16, wherein the gene has a base sequence represented by SEQ. ID. No 8 coding the mutant lipase protein ~~of claim 11~~ wherein the #219 leucine is replaced by a hydrophilic amino acid selected from a group consisting of glutamine, histidine, arginine, lysine, serine, threonine, aspartic acid and glutamic acid.
18. (currently amended) The gene as set forth in claim 16, wherein the gene has a base sequence represented by SEQ. ID. No 6 coding the mutant lipase protein ~~of claim 13~~ wherein the #278 leucine is replaced by an amino acid selected from a group consisting of proline, tyrosine, phenylalanine, tryptophane and valine.
19. (currently amended) The gene as set forth in claim 16, wherein the gene has a base sequence represented by SEQ. ID. No 7 coding the mutant lipase protein ~~of claim 15~~ wherein the #219 leucine is replaced by glutamine, the #278 leucine is replaced by proline, and its amino acid sequence is represented by SEQ. ID. No 10.
20. (original) An expression vector containing the gene of claim 16.
21. (currently amended) The expression vector as set forth in claim 20, wherein the vector is composed of a promoter gene, a secretion signal sequence gene, ~~a gene of claim 17,~~ a gene having a base sequence represented by SEQ. ID. No 8 coding the mutant lipase protein wherein the #219 leucine is replaced by a hydrophilic amino acid selected from a group consisting of glutamine, histidine, arginine, lysine, serine, threonine, aspartic acid and glutamic acid, a terminator gene and/or a surface display-mediating gene.
22. (currently amended) The expression vector as set forth in claim 20, wherein the vector is composed of a promoter gene, a secretion signal sequence gene, ~~a gene of claim 18,~~ a gene having a base sequence represented by SEQ. ID. No 6 coding the mutant lipase protein wherein the #278

Attachment A

leucine is replaced by an amino acid selected from a group consisting of proline, tyrosine, phenylalanine, tryptophane and valine, a terminator gene and/or a surface display-mediating gene.

23. (currently amended) The expression vector as set forth in claim 20, wherein the vector is composed of a promoter gene, a secretion signal sequence gene, ~~a gene of claim 19,~~ a gene having a base sequence represented by SEQ. ID. No 7 coding the mutant lipase protein wherein the #219 leucine is replaced by glutamine, the #278 leucine is replaced by proline, and its amino acid sequence is represented by SEQ. ID. No 10, a terminator gene and/or a surface display-mediating gene.
24. (currently amended) The expression vector as set forth in ~~any of claims 21—23~~ claim 21, wherein the promoter gene is selected from a group consisting of GAPDH, PGK, ADH, PHO5, GAL1, GAL10, SED1, MOX, TEF and TPI.
25. (currently amended) The expression vector as set forth in ~~any of claims 21—23~~ claim 21, wherein the secretion signal sequence gene is selected from a group consisting of MF α , PHO5, SUC2, AMY, SED and killer toxin.
26. (currently amended) The expression vector as set forth in ~~any of claims 21—23~~ claim 21, wherein the surface display-mediating gene is selected from a group consisting of SED1, PIR2, TIP1, CWP1, GAS1 and WSC1.
27. (original) A transformant in which the expression vector of claim 20 is introduced.
28. (currently amended) The transformant as set forth in claim 27, wherein the expression vector ~~of claim 22~~ composed of a promoter gene, a secretion signal sequence gene, a gene having a base sequence represented by SEQ. ID. No 6 coding the mutant lipase protein wherein the #278 leucine is replaced by an amino acid selected from a group consisting of proline, tyrosine, phenylalanine, tryptophane and valine, a terminator gene and/or a surface display-mediating gene is introduced (Accession No: KCTC 10320BP).

Attachment A

29. (currently amended) The transformant as set forth in claim 27, wherein the expression vector ~~of claim 23~~ composed of a promoter gene, a secretion signal sequence gene, a gene having a base sequence represented by SEQ. ID. No 7 coding the mutant lipase protein wherein the #219 leucine is replaced by glutamine, the #278 leucine is replaced by proline, and its amino acid sequence is represented by SEQ. ID. No 10, a terminator gene and/or a surface display-mediating gene is introduced (Accession No: KCTC 10321BP).
30. (currently amended) A method for producing the mutant lipase protein of claim 10 by cultivation of ~~the a transformant of claim 27~~ in which an expression vector containing a gene coding the mutant lipase protein is introduced.
31. (original) The method as set forth in claim 30, wherein the culture temperature is 2°C - 20°C lower than the proper temperature of host cell culture.
32. (original) The method as set forth in claim 31, wherein the culture temperature is 25°C - 35°C as the transformant is *Hansenula*.
33. (original) The method as set forth in claim 31, wherein the culture temperature is 20°C - 28°C as the transformant is *Saccharomyces*.